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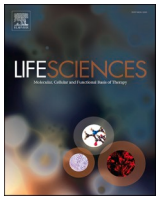

Linking necroptosis with liver aging and chronic inflammation in hepatic pathology

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ABSTRACT

Necroptosis, a regulated form of cell death distinct from apoptosis and necrosis, is increasingly recognized for its role in chronic inflammation and tissue damage within the liver. It is mainly associated with upregulation of necroptotic factors like phosphorylated MLKL (Mixed lineage kinase domain-like protein), Receptor Interacting Kinase 1 (RIPK1), and Receptor Interacting Kinase 3 (RIPK3). The regulation of necroptotic signaling becomes increasingly impaired as liver age progresses, promoting a pro-inflammatory hepatic environment that contributes to the onset and development of age-related liver pathologies such as steatosis, fibrosis, and hepatocellular carcinoma (HCC). The chronic low-grade inflammation associated with aging, often termed "inflammaging," further amplifies necroptosis-mediated damage, establishing a vicious cycle of cell death and inflammatory signaling. Thus, understanding the mechanisms of necroptosis in the aging liver highlights new potential therapeutic targets to alleviate liver diseases. Current therapeutic strategies include the development of small molecule inhibitors that target crucial components of the necroptotic pathway, such as inhibitors of RIPK1 (e.g., Necrostatin-1), RIPK3, and MLKL blockers. The present review delves into the complex role of necroptosis in liver aging and chronic liver diseases, detailing the underlying mechanisms and the latest treatment approaches, and underscores the critical need for extensive research in the area.

1. Introduction

Liver aging is characterized by several degenerative changes which often affect both liver structure and its function that lead to reduced metabolic capacity, impaired regeneration, and increased susceptibility to diseases. It includes mitochondrial dysfunction, accumulation of oxidative stress, DNA damage, impaired autophagy leading to reduced hepatocyte proliferation and altered immune responses, and cellular senescence which lowers the liver function [1]. Several regulatory cell death mechanisms like pyroptosis, ferroptosis, apoptosis, and necroptosis play critical role in liver aging and age-related hepatic diseases. Pyroptosis, ferroptosis, apoptosis and necroptosis are distinct forms of regulated cell death, each characterized by unique molecular mechanisms and biological consequences. Pyroptosis is a caspase-dependent, pro-inflammatory cell death pathway, primarily mediated by caspase-1 (and sometimes caspase-4/5/11), which cleaves gasdermin D to form membrane pores, resulting in cell lysis and the release of pro-inflammatory cytokines such as IL-1β and IL-18 [2]. Apoptotic cell death involves limited release of DAMPs which results in minimal inflammation. Caspase-8 is the key initiator of apoptosis and its absence immediately switches the cell death pathway from apoptosis to necroptosis [3]. Ferroptosis, on the other hand, is an iron-dependent, non-apoptotic cell death process characterized by the accumulation of lipid peroxides to lethal levels, leading to oxidative membrane damage and

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cell death.

Necroptosis is a programmed, caspase-independent form of necrotic cell death that is highly inflammatory [4]. This age-associated upsurge in necroptosis is linked to "*inflammaging*," which is characterized by low-grade chronic inflammation that becomes more evident with age [5]. The necroptotic signaling mechanisms have likely evolved as an alternative mechanism to eliminate virus-infected cells while promoting inflammatory and immune responses to inhibit viral spread [3]. Inflammaging is marked by elevated levels of circulating pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β). Increased concentrations of these cytokines are linked to the development of diseases and higher mortality rates [6]. Several types of cells present in liver including hepatocytes, Kupffer cells, and endothelial cells have been reported to undergo necroptosis in certain pathological conditions or injury [1]. Inflammaging results from complex interactions involving multiple organs and immune cells, particularly macrophages in tissues such as adipose tissue, liver, and kidneys, play a key role in producing these pro-inflammatory cytokines [7].

Necroptosis is initiated by specific necroptotic stimuli that sequentially activate receptor-interacting protein kinase 1 (RIPK1) and 3 (RIPK3) through phosphorylation. These activated kinases then phosphorylate the pseudo kinase mixed lineage kinase domain-like (MLKL) protein, initiating the necroptotic process through the release of damage-associated molecular patterns (DAMPs). These DAMPs activate PRRs (Pattern recognition receptors) like TLRs (Toll-like receptors) on immune cells, promoting pro-inflammatory cytokine production markers (such as TNFα, IL1β, and IL6) and macrophage activation within the liver [8] [9].

Although necroptosis has been implicated in the pathophysiology of aging-related disorders including neurodegenerative, renal, cardiovascular, and pulmonary diseases, its specific role in hepatic aging and the progression of liver diseases remains underexplored. Targeting necroptosis offers potential therapeutic opportunities to reduce liver damage in aging as well as in the prevention of various liver-related disorders. Current research strategies primarily target the development of small molecule inhibitors aim at key components of the necroptotic pathway, particularly RIPK1 and RIPK3. The individual areas of necroptosis in liver disease, liver aging, necroptosis and inflammation have been reviewed extensively. However, limited literature is available which examines the connection of necroptosis and liver aging, and how this interaction contributes to age-related liver disease progression. Our manuscript emphasizes the emerging evidence of linking necroptosis with liver aging through various age-related mechanism and cell-type specific roles, and translational potential of the small molecule inhibitors. This review aims to elucidate the intricate mechanisms of necroptosis in liver aging, its regulatory pathways, and emerging therapeutic approaches in progression of chronic liver diseases.

2. Methodology

A comprehensive literature review was carried out using several scientific databases, including *PubMed*, *ScienceDirect*, *SpringerLink*, *Scopus*, and *Google Scholar*. The search strategy employed specific keywords and MeSH terms such as "Necroptosis," "Liver aging," "Inflammation," "Chronic liver disease," "Therapeutic strategies," "RIPK1 inhibitors," "RIPK3 inhibitors," and "MLKL inhibitors," combined with Boolean operators (AND, OR, NOT) to refine the results. Only peer-reviewed articles from reputable journals indexed in Scopus or PubMed were considered, ensuring the quality and reliability of the sources. Inclusion criteria were limited to English-language studies that focused specifically on liver aging, necroptosis, and related therapeutic strategies.

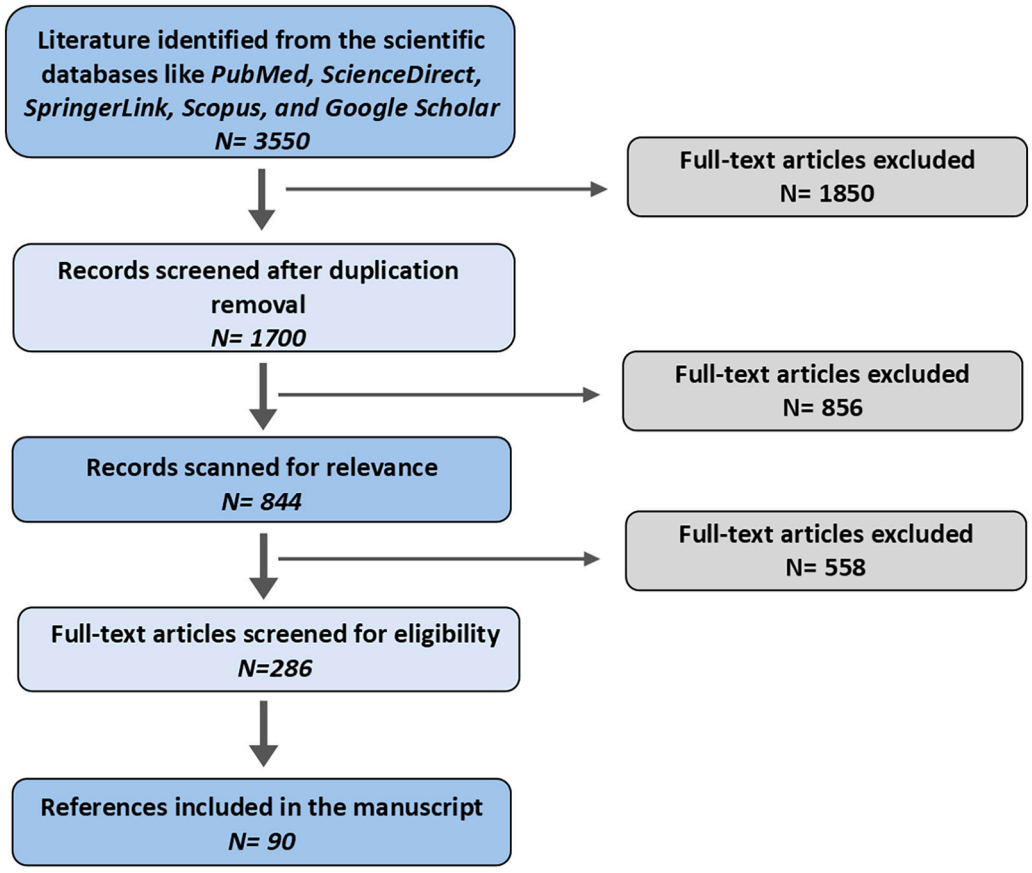

Fig. 1. Represents the pictorial illustration of data collected from various scientific databases like *ScienceDirect*, *PubMed*, *Scopus*, and *Google Scholar*. The review includes 90 full-text articles selected after screening and exclusion criteria.

Exclusion criteria included conference abstracts, books and book chapters, unpublished manuscripts, non-English publications, articles without full-text access, studies unrelated to necroptosis and liver aging, and duplicate records identified during the screening process (Fig. 1).

2.1. Molecular mechanisms of necroptosis: insights into canonical and non-canonical signaling pathways

2.1.1. Canonical pathway of necroptosis

Necroptosis, a regulated type of necrotic cell death marked by cellular swelling and loss of plasma membrane integrity, occurs when apoptosis is inhibited. Apoptosis is an irreversible process in which caspase activation commits a cell to death, making its regulation highly controlled. The two primary methods by which caspase is activated are the extrinsic (death receptor-dependent signaling mechanism) and intrinsic (mitochondria-dependent signaling mechanism) pathways. Signals like TNF signaling or Fas ligand (FasL) activating Fas receptors belong to extrinsic cell death mechanism [10]. Necroptosis mainly involves the binding of ligands to death receptors, including as Fas/CD95, tumor necrosis factor receptor (TNFR)1, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors [11]. The binding of TNF-α to its receptor, TNFR1, initiates the classic necroptotic signaling cascade. This interaction leads to the formation of "Complex I," a multiprotein assembly comprising the linear ubiquitin chain assembly complex (LUBAC), cellular inhibitors of apoptosis (cIAP1 or cIAP2), RIPK1, TNF receptor-associated factors 2 and 5 (TRAF2/5), and TNFR-associated death domain (TRADD) [12].

Complex I play a dual role and it can promote cell survival through NF-κB signaling or initiate apoptosis. TRADD serves as an adaptor, recruiting RIPK1 to TNFR1. The stability and activation of Complex I depend on the ubiquitination of RIPK1, facilitated by cIAPs, TRAF2/5, and LUBAC. This ubiquitination process enables the recruitment of downstream signaling molecules, such as TGF-activated kinase 1 (TAK1), TAK1-binding proteins 2 and 3 (TAB2/3), and the IκB kinase (IKK) complex (comprising IKKα, IKKβ, and NF-κB essential modulator [NEMO]). These downstream proteins activate NF-κB and MAPK signaling pathways, promoting cell survival. However, in certain conditions, Complex I become unstable as a result of the deubiquitination of RIPK1, which is facilitated by enzymes such as CYLD and A20. The deubiquitination results in the formation of cytosolic apoptotic complexes like Complex IIa and Complex IIb, consisting of Caspase-8 and Fas-associated death domain protein (FADD). RIPK1 consequently separates from Complex I and interacts with FADD and Caspase-8, resulting in apoptotic cell death [11] [12].

When Caspase-8 is inhibited or inactivated, RIPK1 interacts with RIPK3 via their RIP homology interaction motifs (RHIM), forming RIP homotypic interactions. RIPK1 can undergo auto-phosphorylation at S166 and bind to RIPK3 through these RHIM domains, leading to the assembly of the necrosome (complex IIb). The pseudokinase domain of MLKL is phosphorylated at Thr357 and Ser358 by the necrosome complex. Because of the conformational shift caused by this phosphorylation, the 4-helical bundle (4HB) domain is exposed, promoting MLKL oligomerisation. The oligomerized MLKL is then transported to the plasma membrane via the Golgi-microtubule-actin network. Once at the membrane, it forms pore clusters in association with tight junction proteins, ultimately leading to membrane rupture and necroptosis [13] [11] [14]. In hepatocytes, the RIPK3 expression is suppressed via epigenetic regulation primarily through different mechanisms like DNA methylation, histone modifications, microRNAs, and chromatin remodelling. These epigenetic regulations help in controlling necroptotic cell death in hepatocytes. The silencing of RIPK3 makes the cells inherently resistant to necroptosis under normal physiological conditions. The suppression is more likely to occur through DNA methylation and/or the addition of repressive histone marks at its gene locus. As the liver ages, these regulatory mechanisms become dysregulated and results in the overexpression of necroptotic-associated genes like RIPK1, RIPK3, and MLKL and enhance susceptibility to cell death [15] (Fig. 2).

2.1.2. Non-canonical pathway of necroptosis

The activation of RIPK1, RIPK3, and MLKL proteins drives cell swelling and subsequent plasma membrane disruption through the previously discussed canonical necroptotic pathway [16]. Besides death receptors, various plasma membrane-bound receptors and intracellular sensors, such as toll-like receptors (TLR3 and TLR4), TRIF, and DAI (DNA activator of interferon) can also trigger signaling cascades that ultimately activate MLKL, leading to MLKL-mediated necroptosis. These proteins contain RHIM domains, which form a non-classical necrosome and execute necroptosis via a non-canonical signaling pathway [11] [16]. The double-stranded RNA (dsRNA) activates TLR3, while lipopolysaccharide (LPS) activates TLR4, which in turn causes necroptosis via TRIF-RIPK3 interaction that is dependent on the RHIM domain. Similar to RIPK1 and RIPK3, caspase-8 cleaves TRIF. Although the role of RIPK1 is yet unknown, TRIF-mediated necroptosis requires its RHIM domain to connect with RIPK3 [14]. The ZBP1 (*Z*-DNA-binding protein 1, often referred to as DAI or DLM-1) is another RHIM-domain-containing protein when activated by viral nucleic acids induce RIPK3- and MLKL-dependent necroptosis independently of RIPK1. Similarly, necroptosis driven by interferons (IFNs) does not rely on RIPK1 kinase activity. Rather, a RIPK1-RIPK3 complex is formed as a result of transcription that is dependent on Jak1/STAT1. Furthermore, IFNs activate the RNA-responsive protein kinase PKR, which interacts with RIPK1 to promote necrosome assembly and initiate necroptosis [17] (Fig. 3).

2.2. Necroptosis in liver aging: underlying mechanisms of age-related changes in liver disease progression

Liver aging is associated with gradual decrease in the regenerative capability of liver subjected to hepatic injury and a predisposition to develop chronic liver diseases such as MASLD, fibrosis and hepatocellular carcinoma (HCC) [18]. Several reports indicate that necroptosis plays a crucial role in mediating age-related hepatic dysfunction. Necroptosis is termed as a form of cell death pathway facilitated by necroptotic factors like RIPK1, RIPK3, and MLKL. The activation of RIPK1/RIPK3 phosphorylates MLKL and transports to the plasma membrane disrupting the membrane integrity and causing cell lysis [14]. Unlike other regulated cell death pathways (apoptosis), necroptosis is highly inflammatory due to release of DAMPs and other inflammatory cytokines (e.g., TNF-α, IL-6), indicating that necroptosis not only contributes to hepatocyte death but also unfolds a pro-inflammatory environment and thus contributing to chronic inflammation—a hallmark of aging known as "inflammaging" [9].

The inflammaging response in necroptosis further aggravates hepatic senescence, mitochondrial dysfunction, epigenetic alterations, increased ER (endoplasmic reticulum) stress and altered intercellular communication [19]. A certain number of in vivo disease models have depicted to the direct corelation of necroptosis to liver aging. For example, in aged mice, the genetic knockdown of RIPK3/MLKL significantly attenuated liver injury, reduced inflammation, and improved metabolic profiles [20]. Also, studies suggests that hepatocyte-specific overexpression of RIPK3 in younger mice led to features mimicking aged livers, including increased fibrosis, steatosis, and immune cell infiltration [5]. These findings emphasise the role of necroptosis in promoting age-related hepatic pathology.

2.2.1. Hepatic cellular senescence

Cellular senescence, a crucial factor in liver aging, is the ability of cells to undergo a permanent exit from the cell cycle in response to cellular damage and stress [21]. In hepatocytes, the cellular senescence involves permanent cell cycle arrest usually triggered by DNA damage and/or activation of p53/p21 pathway which further involves upregulation of p21 and p16 proteins [22]. In aged hepatocytes, deposit the

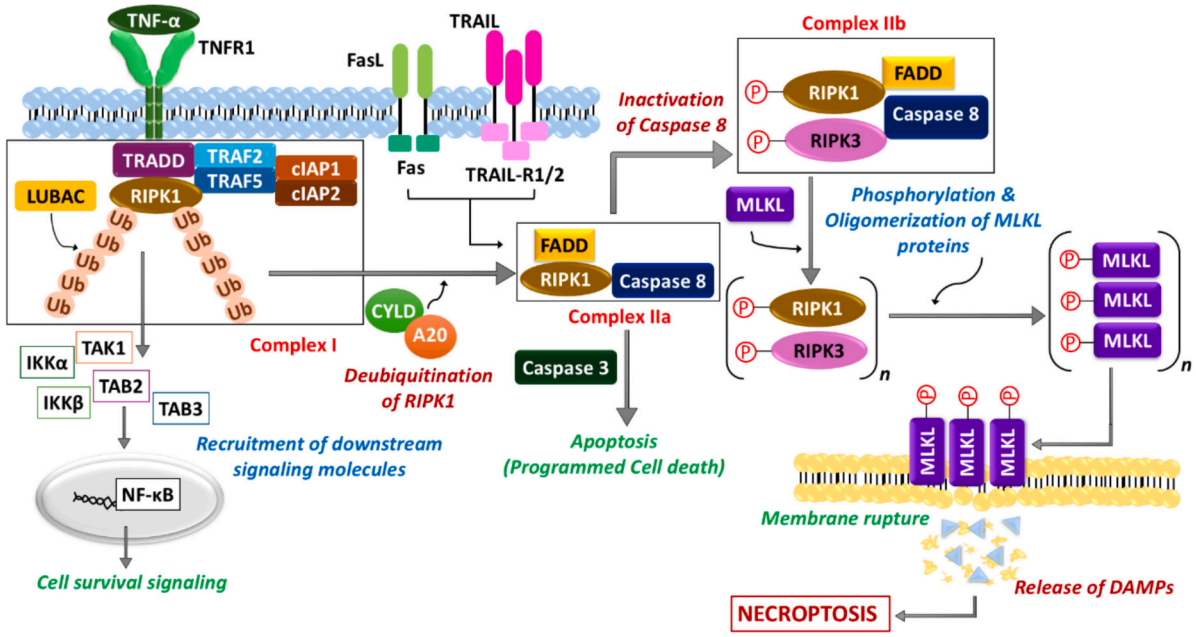

Fig. 2. Illustrates the canonical necroptotic signaling pathway, which involves death receptors such as TNFR1, FasL, and TRAIL. The process begins when TNF-α binds to TNFR1, leading to the formation of "Complex I." The stability and activity of Complex I rely on the ubiquitination of RIPK1 by cIAPs, TRAF2/5, and LUBAC. This ubiquitination helps recruit downstream signaling proteins that activate NF-κB and MAPK pathways, promoting cell survival. However, under specific conditions, RIPK1 undergoes deubiquitination by enzymes like CYLD and A20, which destabilizes Complex I. This destabilization triggers the formation of cytosolic apoptotic complexes, Complex IIa and Complex IIb, which contain FADD and Caspase-8. If Caspase-8 is inactive, RIPK1 interacts with RIPK3 through their RHIM, leading to the formation of Complex IIb. Complex IIb facilitates the oligomerization of MLKL, which is then transported to the plasma membrane via the Golgi-microtubule-actin network. Once at the membrane, MLKL forms pore clusters in coordination with tight junction proteins, ultimately causing membrane rupture and inducing necroptosis.

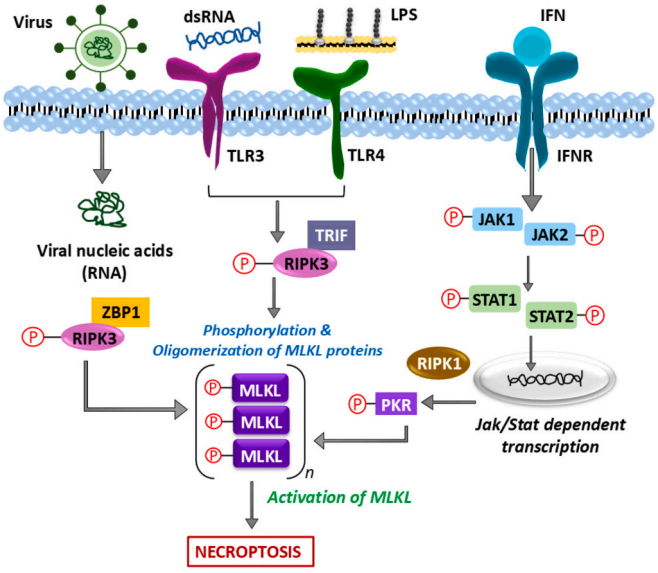

Fig. 3. Illustrates the non-canonical signaling pathways of necroptosis. In addition to death receptors, various plasma membrane-bound receptors and intracellular sensors, such as TLR3, TLR4, TRIF, and DAI, can initiate necroptotic signaling. TLR3 and TLR4, activated by dsRNA and LPS, respectively, induce necroptosis via RHIM domain-dependent interactions with RIP3. Viral nucleic acids activate ZBP1, which triggers RIP3-MLKL-dependent necroptosis without RIP1. Additionally, IFN signaling via JAK/STAT can induce necroptosis, partly by activating PKR, which phosphorylates RIP1. Despite diverse triggers, all pathways ultimately converge on MLKL activation, the key effector of necroptosis.

senescent cells which produce a characteristic pathogenic senescence-associated secretory phenotype (SASP) which includes TNF-α, a potent inducer of necroptosis via the TNFR1–RIPK1–RIPK3–MLKL pathway. Elevated levels of SASP further promote necroptotic cell death and release DAMPs which aggravates the inflammaging in hepatocytes [9]. Moreover, pharmacological agents like Necrostatin-1 or genetic deletion of RIPK3/MLKL proteins was found to reduce the senescence markers in mice [5]. The MLKL proteins, independent of their role in necroptosis, exert anti-senescence effects in hepatocytes. The upregulation of hepatocyte-specific MLKL protein expression induce mitochondrial dysfunction, oxidative stress, extracellular vesicle release, and senescence marker expression which features novel, non-lethal mechanism connecting necroptosis machinery to cellular aging [23]. Senolytic agents such as Dasatinib and Quercetin selectively eliminate senescent cells, suppress SASP factors like TNF-α, and reduce necroptosis in Superoxide Dismutase 1 (SOD1) knockout mice. Additionally, pharmacological inhibitors like Necrostatin-1 have been shown to decrease key senescence markers, including p16, p21, and p53 [24].

2.2.2. *Mitochondrial dysfunction*

In aged hepatocytes, alterations in the mitochondrial functions due to mutations in mitochondrial DNA, increased oxidative stress, disrupted oxidative phosphorylation, and structural abnormalities have impaired the cellular energy metabolism and elevated the oxidative stress that drives cellular senescence [25]. Mitochondrial dysfunction drives necroptosis in aging liver through increase in reactive oxygen species (ROS) and promotes synthesis of pro-inflammatory markers (e.g., TNF-α, IL-6). The necrosome complex enhances the mitochondrial oxidative phosphorylation which in turn augments RIPK1 autophosphorylation and promote necroptosis [26]. Furthermore, activation of MLKL protein can trigger mitochondrial damage through PGAM5 (phosphoglycerate mutase family member 5) and Drp1 (dynamin-related protein 1)-mediated fragmentation and mitochondrial

permeability transition pore (MPTP) opening that involves cyclophilin D [27]. In the absence of cell death, hepatocyte-specific MLKL overexpression in aged mouse liver causes oxidative stress, mitochondrial structural abnormalities, disrupted bioenergetics, and SASP secretion, which suggests the non-necroptotic role for MLKL in promoting inflammation and mitochondrial dysfunction [23]. Altogether the above findings depicts that mitochondrial dysfunction plays a major role in exacerbating necroptosis in hepatocytes which drives the chronic liver disease conditions.

2.2.3. Epigenetic alterations

Multi-omics studies represent that both genomic and epigenetic alterations are associated with the dysregulation of necroptotic-related genes in liver. DNA methylation and histone modifications are abnormal type of epigenetic alterations correlated with altered expressions of necroptotic factors in liver aging mechanism [28]. Epigenetic reprogramming, on the other hand, is a significant contributor to liver aging: Hepatocyte metabolism, regeneration, and genomic stability are hindered by alteration in histone modification, chromatin remodelling (such as C/EBPα–HDAC1/Brm complexes in the livers of aged mice), DNA methylation shifts (including age-associated promoter hypermethylation), and altered noncoding RNA expression [29]. Furthermore, epigenetic silencing of RIPK3 protects hepatocytes against necroptosis. Preston et al. reported that, methylation-mediated reduction of RIPK3 gene expression prevents MLKL-driven necroptotic death [15]. Thus, the interaction of necroptotic signaling and epigenetic regulation during liver aging orchestrates fibrotic progression, cellular senescence, and inflammation, highlighting potential therapeutic approaches that target both pathways.

2.2.4. Endoplasmic reticulum (ER) stress

ER stress is produced in response to stress signals generated by Heat shock proteins (HSPs) that leads to accumulation of damaged proteins and loss of proteostasis. Aggregation of HSP in liver cells promote promotes the unfolded protein response (UPR), a highly conserved protective response that maintains cellular homeostasis Age-related deactivation of the UPR in the endoplasmic reticulum and mitochondria of hepatocytes is well documented, and has been shown to cause accumulation of misfolded proteins [1]. A growing number of evidence suggests that ER stress plays a significant role in the progression of necroptotic pathway during liver aging which exacerbates inflammation and tissue damage. Zhon et al. reported increase in ER stress and elevated liver ischemia and reperfusion (IR) injury in aged mice. ER stress upregulated the expression of glucose-regulated protein 78 (GRP78) in aged livers than in younger livers, particularly after ischemia-reperfusion (IR) stress which indicates that hepatocytes are more prone to necroptosis through RIPK1/RIPK3/MLKL signaling pathway, which causes membrane disruption, DAMP release, and increased inflammatory injury. Also, the IR-induced necroptosis in old mice is attenuated by pharmacologically suppressing ER stress with 4-phenylbutyrate (4-PBA), demonstrating that ER stress closely associated with necroptosis [30] (Table 1).

2.3. Necroptosis in liver aging: cell-specific roles in hepatocytes, Kupffer cells, and stellate cells

Liver aging is marked by progressive changes in the cell homeostasis, regeneration capacity and immune regulation. Necroptosis implies a regulated form of necrosis mediated by RIPK1, RIPK3, and MLKL. The effect of these necroptotic factors may vary and depend on the liver cell type, with diverse outcome on Hepatocytes, Kupffer cells, and hepatic stellate cells (HSCs).

2.3.1. Hepatocytes

Hepatocytes are considered as the principal parenchymal cells in liver responsible for major hepatic functions like biosynthesis of albumin, fibrinogen, and lipoproteins; regulation fatty acid and carbohydrate metabolism; synthesize of cholesterol and bile salts; and xenobiotic metabolism [1]. In normal conditions, hepatocytes generally suppress necroptosis by silencing the key necroptotic components (RIPK1, RIPK3, and MLKL) through DNA methylation of the RIPK3 promoter. This prevents the liver cells from undesirable cell death and pro-inflammatory cytokine release. But during liver aging or any diseased states, there is upregulation of RIPK3 protein expression in hepatocytes that eventually release DAMPs and produce liver inflammation and injury [31]. Additionally, necroptosis in hepatocytes is implicated in the progression of chronic liver diseases such as non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), where it aggravates hepatic injury and fibrosis [32].

2.3.2. Kupffer cells

Kupffer cells are resident macrophages situated within the lumen of liver sinusoids which readily undergo necroptosis when compared to hepatocytes in response to certain inflammatory stimuli or any infections. Liver aging in Kupffer cells are correlated with increased inflammation and necroptosis which release DAMPs and pro-

Table 1
The tabular column summarizes the four key mechanisms (*Hepatic cellular senescence*, *Mitochondrial Dysfunction*, *Epigenetic Alterations*, *Endoplasmic Reticulum Stress*) in liver aging and their connections with necroptosis.

Sl. No.	Mechanism	Key-features in liver aging	Connection to necroptosis	References
1	Hepatic cellular senescence	• Permanent cell cycle arrest via DNA damage and p53/p21 pathway • Upregulation of p21, p16 • SASP production (e.g., TNF-α).	• SASP cytokines (e.g., TNF-α) activate *TNFR1–RIPK1–RIPK3–MLKL* necroptotic pathway • DAMP release from necroptotic cells worsens inflammaging. • Necrostatin-1 and RIPK3/MLKL inhibition reduce senescence MLKL promotes mitochondrial dysfunction and senescence markers independent of cell death	[21]; [22]; [9]; [5]; [23]; [24]
2	Mitochondrial dysfunction	• Mitochondrial DNA (mtDNA) mutations, ROS accumulation. • Disrupted oxidative phosphorylation. • Impaired bioenergetics and structure	• ROS enhances pro-inflammatory cytokines • RIPK1 auto-phosphorylation promoted by mitochondrial oxidative stress • MLKL activation causes mitochondrial fragmentation and MPTP opening. • MLKL-induced mitochondrial stress contributes to SASP and inflammation	[25]; [26]; [27]; [23]
3	Epigenetic alterations	• DNA methylation • Histone modification • Chromatin remodelling (e.g., C/EBPα–HDAC1/Brm) • Altered noncoding RNA expression	• Epigenetic silencing (e.g., RIPK3 methylation) protects against necroptosis. • Changes in chromatin structure affect expression of necroptotic genes. • Crosstalk promotes senescence, fibrosis, and inflammation.	G. [28]; [29]; [15]
4	Endoplasmic Reticulum (ER) stress	• Accumulation of misfolded proteins • Impaired UPR in aged hepatocytes • Upregulation of GRP78 after stress	• ER stress activates RIPK1/RIPK3/MLKL pathway. • Promotes membrane disruption and DAMP release. • 4-PBA suppresses ER stress and reduces necroptosis in aged livers	[1]; [30]

inflammatory cytokines, perpetuating a pro-inflammatory microenvironment that accelerates liver aging and fibrosis. Also, necroptosis in KCs helps balance pro-inflammatory (type 1) and tissue-repair (type 2) responses, influencing the outcome of liver injury and repair during infections caused by *Listeria monocytogenes* [33,34].

2.3.3. Hepatic stellate cells (HSCs)

HSCs are pericytes located in the Space of Disse having crucial role in Vitamin A and lipid storage, and extracellular matrix production. Upon activation HSCs lose their characteristic properties and transforms into myofibroblasts which drives the fibrosis progression [35]. HSCs play a central role in liver fibrosis and express necroptotic mediators such as MLKL and RIPK3. The activation of MLKL molecule in HSCs induce epithelial-mesenchymal transition (EMT), further promoting HSC activation and fibrogenesis independently of its role in cell death. Also, interestingly, MLKL is reported to have non-necroptotic roles in HSCs, influencing their activation and the fibrotic process through transcriptional regulation, further linking necroptosis to liver aging and chronic disease progression [36].

2.4. Role of necroptosis in liver aging and its impact on chronic liver disease progression

Liver aging is marked by a series of degenerative changes that impair its function and reduce its regenerative capacity. With age, the liver undergoes a decline in functional mass, leading to metabolic disturbances and heightened susceptibility to disease [37]. Recent studies highlights necroptosis, a regulated form of programmed cell death, as a potential therapeutic target for aging-related liver disorders. This connection is particularly relevant in the progression of age-associated conditions such as chronic liver disease and HCC [38]. Necroptosis not only contributes to hepatocyte death but also fuels inflammation that drives disease advancement. Concurrently, aging exacerbates these processes through increased necroptotic activity and diminished regenerative capabilities. Understanding these interactions opens potential pathways for targeted therapies aimed at mitigating liver damage in aging populations suffering from chronic liver diseases [39] (Table 2).

2.4.1. Non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH)

Formerly known as Non-Alcoholic Fatty Liver Disease (NAFLD), Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a chronic liver disease that is defined as an excessive accumulation of lipid in the liver, usually without significant alcohol consumption. A consensus among healthcare professionals led to the official acceptance of the new nomenclature, MASLD, in 2023 with the goal of lowering stereotypes and more accurately reflecting the disease's metabolic foundations [47] [48]. The progression from NAFL to NASH is influenced by various factors, including necroptosis and the aging process of the liver. Necroptosis is implicated in the progression of NAFLD towards NASH by promoting inflammation and fibrosis. Factors such as lipotoxicity and oxidative stress can trigger necroptotic cell death, which feeds into a cycle of inflammation that perpetuates liver damage [17]. The classic canonical necroptotic signaling primarily involves TNFR1 activation through TNF-α and thereby stimulating the downstream signaling molecules like RIPK1, RIPK3, and MLKL. The RIPK1-RIPK3-MLKL complex translocates into the plasma membrane causing rupture and release of DAMPs [11]. Increased expression of necroptosis-related proteins, particularly RIPK3 and MLKL, has been observed in liver tissues from NAFLD patients. NAFLD may develop due to a multitude of signaling pathways, including metabolic and inflammatory mediators, which can trigger necroptosis.

Polarity proteins are class of proteins that constitute both transmembrane and scaffold proteins. The scaffold protein AF6 is widely expressed in epithelial tissues. Studies show that AF6 is essential for preserving blood glucose homeostasis and improving the liver's sensitivity to insulin. Dai et al. demonstrated that primary hepatocytes subjected to steatosis induced by oleic acid (OA) and palmitic acid (PA) exhibit a direct upregulation of AF6 expression [49]. AF6 can directly bind to the intermediate domain of RIPK1 and regulate its ubiquitination through deubiquitylase enzyme USP21, which accelerates the development of NAFLD. Hepatocyte-specific AF6 deletion, thus, prevented necroptosis and the resulting inflammation in a number of non-alcoholic liver diseases, whereas overexpression of AF6 results in TNFα-induced necroptosis-mediated liver cell death [41]. The MLKL signaling contributes to the pathophysiology of NAFLD by controlling various cell

Table 2
The tabular column provides an overview of the possible contribution of liver aging and necroptosis to the development of chronic liver disorders.

Sl. No.	Disease condition	Target	Main findings	Reference
1	Non-Alcoholic Fatty Liver Disease (NAFLD)/Non-Alcoholic Steatohepatitis (NASH)	RIPK3, MLKL	Increased expression of RIPK3 and MLKL correlates with severity of liver damage, including steatosis and inflammation.	[40]
2	NASH	AF6, RIPK1, USP21	AF6 regulates RIPK1 ubiquitination; its deletion inhibits necroptosis and inflammation, while overexpression promotes TNFα-induced necroptosis in NASH.	[41]
3	NASH	MLKL	MLKL controls cell death mechanisms, including autophagic flux, influencing NAFLD/NASH progression.	[15]
4	Liver aging and NAFLD progression	MLKL RIPK1	Nec-1 reduced cytokines, M1 macrophage markers, and cell senescence in aged mice livers.	[32]
5	HCC progression with aging	SOD MLKL RIPK1	Loss of antioxidant enzyme increased oxidative stress, fibrosis, and MLKL/RIPK3 expression.	[5]
6	NAFLD severity	RIPK3	TNF-α triggers hepatocyte necroptosis, increasing oxidative stress and fibrosis; RIPK3 deficiency reduces fibrosis.	[42]
7	NASH	RIPK1	RIPK1 inhibition restored steatosis, improved mitochondrial respiration, and reduced inflammation.	[43]
8	Acute-on-chronic liver failure (ACLF)	RIPK1	RIPK1 inhibition reduced ACLF severity, hepatocyte damage, and inflammation.	[44]
9	HCC	RIPK1 TRAF2 caspase-8	RIPK1 deletion downregulated TRAF2, impairing caspase-8 and NF-κB activation, impacting HCC development.	[45]
10	HCC	RIPK3/MLKL FADD	miR-675 targets FADD, inducing necroptosis and preventing HCC progression via RIPK3/MLKL axis.	[46]

death mechanisms, including autophagic flux, in addition to necroptosis-related death. Necroptosis and NAFLD can be induced by another effector molecule called FOXO1 (Forkhead box protein O1). This was studied by two different investigations. The first study was reported by Qian et al. demonstrated that Serpina3c loss caused necroptosis and NAFLD through FOXO1 overexpression [50], the second study postulated that FOXO1 causes endoplasmic reticulum stress and necroptosis, and so plays a role in the pathophysiology of the disease [51].

Non-necroptotic signaling occur independent of TNFR1 and comprises alternative signaling mechanisms involving TLR3/TLR4-TRIF-RIPK3, ZBP1, or IFN-PKR-RIPK3 pathways. These pathways still activate RIPK3 and MLKL, leading to necroptosis without RIPK1 involvement. The TRIF (TIR domain-containing adaptor inducing IFN-β) serves as the important adaptor protein for in regulating necroptosis signaling mediated by TLR3 and TLR4. As a crucial component of TLR signaling, TRIF plays an essential role in multiple signaling pathways and pathological conditions [52]. In a study reported by Yang et al. the CDAA diet induced mouse model, TRIF-deficient animal exhibited less liver steatosis compared to TLR4 wild-type mice. The study findings suggest that TRIF-deficient and TLR4-deficient liver cells exhibited less steatosis unlike wild-type hepatocytes. Also, DGAT2 (diacylglycerol acyltransferase 2), a downstream effector protein was known to elevate the lipid accumulation in the liver cells via TLR-TRIF signaling mechanism [53]. Also, TRIF helps in the recruitment of RIPK1 and RIPK3 via RHIM domain and stimulates necroptotic signaling and promote NF-κB mediated inflammation. In a study reported by Afonso et al. states that hepatic RIPK3 is associated with NAFLD severity in mice. RIPK3 knockout mice exhibited decreased CDAA-induced inflammation and fibrosis in mice at both 32 and 66 weeks whereas, wild type-strain developed preneoplastic nodules and displayed increased hepatocellular proliferation. Also, higher levels of RIPK3 expression was found in NASH patients correlated with increased hepatic inflammation and fibrosis [54].

Majdi et al. reported that MLKL and RIPK3 were overexpressed in mice fed with HFD compared to NCD-fed mice. Treatment with RIPA-56, a RIPK1 inhibitor protected hepatocytes from necroptosis in HFD fed mice and thereby lowering hepatic inflammation and fibrosis. RIPA-56 lowered lipid accumulation in liver cells in MLKL-dependent manner in mice fed with HFD. Also, the study findings suggest that inhibition of RIPK1-MLKL axis can exacerbate mitochondrial respiration and increase the β-oxidation in liver cells. Also, the serum concentrations of RIPK1 and MLKL proteins in 35 NAFLD patients were significantly increased. Collectively, these findings indicate that RIPK1 is critically involved in the progression of NAFLD [43]. ZBP1 (*Z*-DNA-binding protein 1) is another important mediator in the non-canonical necroptotic signaling mechanism which triggers metabolic stress and ROS production in NAFLD condition via activation of RIPK1 and MLKL proteins independent of TNF-α pathway [55]. In a study by Liu et al. reported the overexpression of ZBP1 protein via palmitic acid- activated JNK pathway in the steatotic livers. Also, during ischemia/reperfusion injury in steatotic livers, excessive ROS generated facilitates ZBP1 aggregation RIPK1 activation leading to necroptosis and inflammation which subsequently aggravates the liver injury. From the study findings, activation of ZBP1 promote non-canonical necroptosis via oxidative stress and deletion of ZBP1 can inhibit the RIPK1 activation and further ameliorate the steatotic liver damage [56].

2.4.2. *Liver fibrosis & HCC*

Hepatic fibrosis is triggered by various parameters like hepatotoxicity, viral hepatitis, autoimmune and metabolic disorders, NASH, and cholestatic diseases [57]. liver fibrosis can be developed to irreversible condition like HCC due to chronic liver injury and impaired healing. Inflammation recruits macrophages and produce cytokines and chemokines which subsequently activate the hepatic stellate cells (HSCs) myofibroblasts that produce an excess extracellular matrix (ECM) [58]. Multiple cell death mechanisms, such as necrosis, apoptosis, and necroptosis, occur simultaneously in liver disorders, and hepatocyte death is a primary manifestation of hepatic fibrosis [3]. Necroptosis includes characteristics of both apoptosis and necrosis. RIPK1, RIPK3, and MLKL are important regulators of necroptosis that cause membrane rupture and cell death [57].

Given its involvement in both cell death and inflammation, targeting necroptosis presents a potential therapeutic strategy to mitigate liver inflammation and fibrosis in aging populations [5]. Inhibition of necroptosis has demonstrated promising results in reducing liver inflammation. [59]. It was reported that changes in the expression of TNF-α, IL6, and IL1β, as well as indicators of inflammation (TGF-β1, IFN-γ), are correlated with changes in necroptotic signals in the liver. Compared to young mice, the hepatocytes and hepatic macrophages of elderly mice exhibited higher levels of necroptotic markers and proinflammatory cytokines. On the contrary, Nec-1 reduced the cytokines M1 macrophage markers, and cell senescence in the livers of aged mice. Another study indicates that the deletion of antioxidant enzyme, Cu/Zn-superoxide dismutase increased oxidative stress and accelerated HCC progression with aging. Phosphorylated MLKL (P-MLKL) is a potent biomarker in necroptosis signaling. The SOD-compromised ($Sod1^{-/-}$ knock out) mice exhibited higher expressions of p-MLKL in liver cells along with elevated levels of RIPK3 and phosphorylated RIPK3 proteins. Also, various fibrotic markers like transforming growth factor beta (TGFβ), connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF) were increased with upregulated oncogenic transcription factor STAT3 in $Sod1^{-/-}$ knock-out mice [32].

Afonso et al. examined hepatic RIPK3 as an important factor in in vivo experimental models. From the study report, TNF-α triggers hepatocyte necroptosis, which in turn causes RIPK3-dependent oxidative stress. TNF-α, MLKL, and RIPK3 expression levels were elevated in rats fed HFCD and MCD diets. However, RIPK3 deficiency decreased liver fibrosis induced by the MCD diet [60]. Along with this finding, Majdi et al. revealed that RIPK1 inhibition restored steatosis via an MLKL-dependent mechanism that primarily affects mitochondrial respiration and improved NASH features in HFD-fed mice. They found that RIPA-56 suppresses RIPK1 to either prevent or cure liver damage, inflammation, fibrosis, and steatosis in animal models. MLKL knockout of *Mlkl* in fat-loaded AML-12 mouse hepatocytes and RIPA-56 or Necrosulfonamide, a specific inhibitor of human MLKL, were employed. MLKL-KO enhanced β-oxidation and triggered mitochondrial respiration in steatotic hepatocytes. Furthermore, RIPK3-KO animals exhibited increased activity of the liver mitochondrial respiratory chain complexes in experimental NASH, which is correlated with decreased MLKL activation [43].

Kondo et al. reported that RIPK1-mediated cell death is essential, and it has been demonstrated that blocking RIPK1 may be a novel treatment approach against acute chronic liver failure (ACLF) in individuals at risk. Carbon tetrachloride (CT)-induced fibrosis mice were given galactosamine (CCL4/GalN), while bile-duct ligated rats were given LPS. Nec-1 therapy decreased the severity of ACLF by reducing liver, kidney, and brain damage in connection with decreased hepatic and renal cell deaths. CCL4/GalN-induced ACLF in mice had comparable hepatoprotective effects to RIPA56. Deletion of RIPK1 causes TRAF2 to be downregulated in chronic liver diseases like HCC, which hinders the activation of caspase-8 and NF-κB. As a result, the RIPK1/TRAF2/caspase-8 pathway significantly influences the development of HCC [45]. Another key signaling molecules in necroptosis include FADD, RIPK1, RIPK3, and MLKL. Thus, by targeting FADD, miR-675 may cause necroptosis and prevent the progression of HCC through the RIPK3/MLKL axis [46].

In non-canonical pathway, activation of TL3/TLR4-TRIF-RIPK3 pathway can stimulate highly immunogenic form of necroptotic cell death in hepatocytes with chronic inflammation that leads to extracellular matrix production and liver fibrosis progression [54]. Wei et al. reported upregulated RIPK3 protein expression in liver tissue and macrophages of mice with liver fibrosis. The deletion of RIPK3 can

significantly lower the CCl4-induced and BDL-induced liver fibrosis in mice and prevent the TLR4–NF-kB pathway activation through suppressing Rho-associated coiled-coil containing protein kinase (ROCK)1 in macrophages [61]. In cholestatic liver condition like biliary atresia, the ZBP1 overexpression in liver macrophages induced necroptosis through ZBP1/p-MLKL pathway and was correlated with liver fibrosis and prognosis. Also, in mouse model of liver fibrosis (bile duct ligation-induced mice liver injury model) the upregulated ZBP1 and MLKL levels were directly proportional to the fibrosis severity [55]. Selvarani et al. established that feeding RIPK3-knock in and MLKL-knock in mice with western diet exhibited upregulated RIPK3 and MLKL protein expressions compared to control. Also, mice fed with western diet for 12 months produced large tumor nodules with increased mRNA levels of proinflammatory cytokines (TNFα, IFNa2). Taken together, all these findings suggest that inflammation induced by necroptosis can progress liver steatosis to fibrosis and liver cancer [62].

While necroptosis is often unfavourable in chronic disease conditions like MASLD/MASH and fibrosis and cirrhosis, its context-dependent role particularly in HCC serve as a protective role. Interestingly, studies suggest that not all liver diseases are benefited from the inhibition of necroptosis pathway. However, in HCC, necroptosis was found to promote the death of malignant cells signifying the therapeutic role of this pathway in eliminating cancerous liver cells. This dual action of necroptosis in liver cancer progression is not beneficial for other type of chronic liver conditions. However, future research should highlight the therapeutic strategies to employ the antitumor potential of necroptosis while minimizing its inflammatory consequences [33]. At present, the majority of the literature evidence comes from the pre-clinical animal models (primarily mice) with a strong in vivo experimental data linking necroptosis to age-related liver inflammation and fibrosis. There is one clinical report available on necroptosis has been implicated in human primary biliary cholangitis (PBC) [42]. Clinical reports directly linking necroptosis and liver aging in Liver diseases are still emerging. Gautheron et al. reported histological and transcriptomic analyses of liver samples from elderly individuals have shown elevated levels of RIPK1, RIPK3, and MLKL, particularly phosphorylated MLKL (p-MLKL), indicating activation of necroptosis. These increases correlate with inflammatory changes, fibrosis, and hepatocyte loss—hallmarks of aged or diseased livers [84].

2.5. Necroptosis in liver aging and chronic disease: unveiling therapeutic implications

Necroptosis, a regulated form of cell death, has emerged as a significant contributor to liver aging and associated chronic liver conditions, including liver fibrosis, cirrhosis, and HCC [34]. Recent studies indicate that aging is associated with an increase in necroptosis markers, such as phosphorylated MLKL, RIPK1, and RIPK3 leading to intensified inflammation characterized by elevated levels of pro-inflammatory cytokines DAMPs [9]. Targeting necroptosis presents a potential therapeutic strategy for mitigating liver aging effects and associated diseases. *Necrostatin-1* (Nec-1) and *RIPA56* specifically target RIPK1. *Dabrafenib*, primarily a B-Raf inhibitor, has been identified as a potent RIPK3 antagonist. Meanwhile, *Necrosulfonamide* (NSA) is the most widely used MLKL inhibitor in preclinical studies (Fig. 4).

2.5.1. RIPK1 inhibitors

RIPK1 inhibitors have become promising therapeutic agents for a number of liver diseases as it plays a crucial role in regulating necroptosis, a type of cell death linked to liver injury, inflammation, and

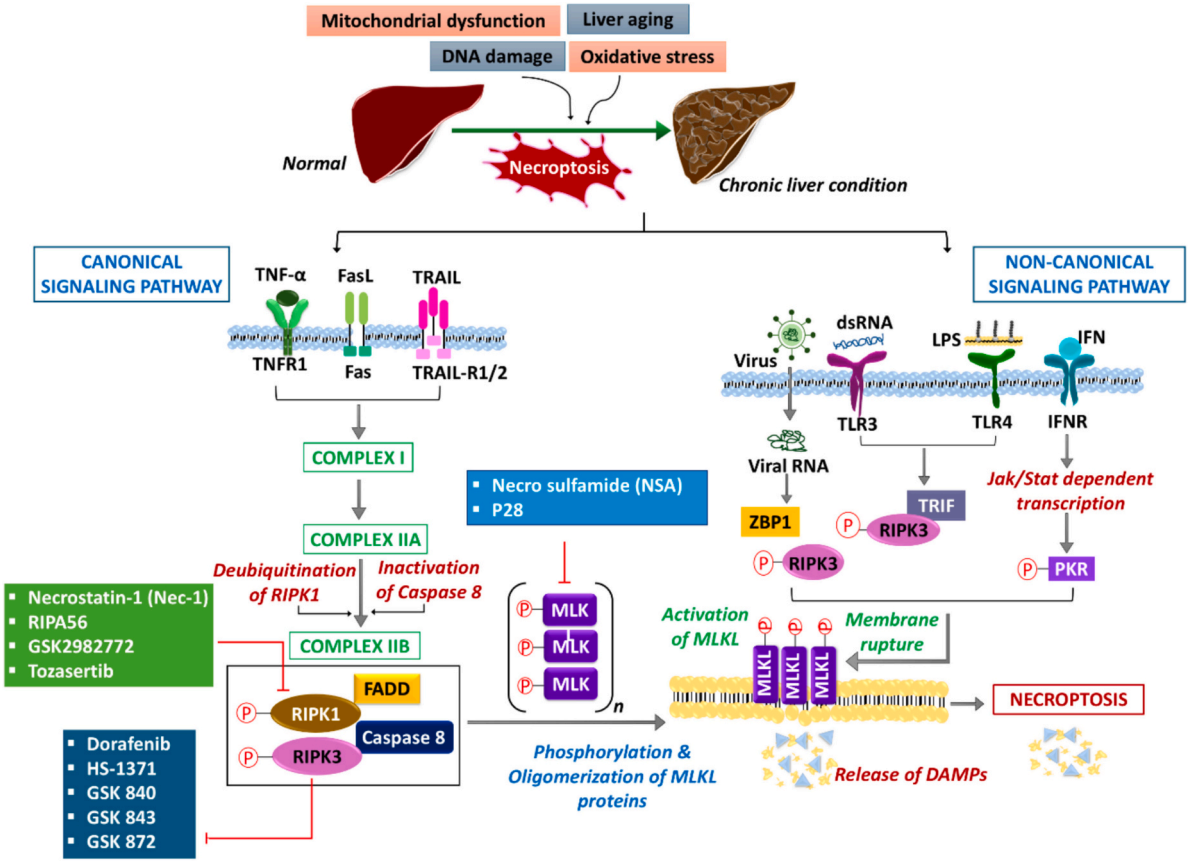

Fig. 4. Depicts the action of potential drug candidates on various necroptotic markers like phosphorylated MLKL and RIPK1, and RIPK3 in the Necroptotic signaling pathway. Necrostatin-1 (Nec-1) and RIPA56 targets the RIPK1. Dabrafenib, a B-Raf inhibitor, is reported to have strong RIPK3 antagonistic activity and Necrosulfonamide (NSA) is the most commonly employed MLKL inhibitor in experimental studies.

fibrosis. Nec-1 or Necrostatin-1 [chemically known as 5-(1H-indol-3-ylmethyl)-2-thiohydantoin 1], initially discovered during a investigating procedure for necroptosis inhibitors was identified as an allosteric inhibitor of RIP1, which stabilizes the inactive conformation of the kinase domain [63]. Also, inhibiting RIPK1 with Nec-1 and RIPA56 (SML2100) prevented the occurrence of ACLF in two in-vivo models, indicating that necroptosis is probably detrimental and a possible target for treatment to prevent the onset of acute-on-chronic liver failure. A more potent derivative, Nec-1s (7-Cl-O-Nec-1), demonstrates increased specificity for RIP1 compared to other kinases. Additionally, several other inhibitors, such as furo [2,3-d] pyrimidines and GSK'963, have been developed as RIP1 inhibitors [64]. It is extensively utilized in disease models to investigate the role of RIP1 in cell death and inflammation.

Other necrostatin variants, such as Nec-3, Nec-4, and Nec-5, also stabilize RIP1 in its inactive conformation by interacting with hydrophobic pockets and highly conserved amino acids [65]. A compound GSK2982772 was discovered as a new RIP1 inhibitor in an earlier investigation. Strong binding affinity for RIP1 is demonstrated by GSK2982772, which also shows exceptional selectivity for the kinase and successfully inhibits TNF-dependent inflammation and necroptosis. Another Compound namely 7-oxo-2,4,5,7-tetrahydro-6H-pyrazolo(3,4-*c*)pyridine) as a new class of RIP1 kinase inhibitors that Yoshikawa et al. designed and synthesized based on the findings regarding GSK2982772. It exhibits moderate inhibitory activity against RIP1 kinase and is susceptible to P-glycoprotein-mediated efflux [66]. Tozasertib, also known as VX-680 or MK-0457, is a pan-Aurora kinase inhibitor that has recently been demonstrated to be an effective agent for preventing cytokinesis in cells and RIP1-dependent necroptosis [67]. In conclusion, the aforementioned studies suggest that RIPK1 can be a potential target for developing newer therapeutic regimens against chronic liver diseases [67].

2.5.2. *RIPK3 inhibitors*

In contrast to RIPK1, RIPK3 is crucial for necroptosis but not for apoptosis, making it a more specific target for controlling necroptosis. Notably, the silencing of RIP3 significantly protects cells and tissues from necroptosis [64]. However, very few specific inhibitors have yet been reported. Dabrafenib, a B-Raf inhibitor, is reported to have strong RIPK3 antagonistic activity. Dabrafenib suppressed the interaction between RIPK3 and MLKL and decreased RIP3-mediated Ser358 phosphorylation of MLKL. Based on these findings, Dabrafenib might act as a RIPK3 inhibitor and be used as a treatment option for liver disorders associated with RIP3-involved necroptosis [68]. Small molecules such as GSK′840, GSK′843, and GSK′872 suppress RIP3-dependent necroptosis. These small molecule inhibitors mechanistically restore caspase activity, indicating that RIPK3 blockers offer cells more protection against a wider range of stimuli than RIP1 inhibitors [69]. Recently, Park et al. recently discovered an effective RIP3 inhibitor, HS-1371, by a thorough screening of chemical databases targeted on kinases. HS-1371 binds directly to RIP3 in a time-independent and ATP-competitive approach. Additionally, the molecule reduced TNF-induced necroptosis but not TNF-induced apoptosis, suggesting that this new inhibitor specifically blocks RIP3-mediated necroptosis by suppressing RIP3 kinase activity [70].

Recently, Mohammed et al., showed that RIPK3 knockout in aged mice worsens glucose intolerance and shortens lifespan [32]. Similarly, Roychowdhury et al. also reported glucose intolerance in RIPK3-deficient models under diet-induced stress [71]. These findings do not fully support the idea that RIPK3 inhibition is a good therapeutic strategy for aging or liver disease. A recent study by Mohammed et al. [20] shows that deleting MLKL or RIPK3 reduces age-related liver inflammation in MASLD. However, MLKL knockout improves insulin sensitivity, while RIPK3 knockout leads to glucose intolerance, highlighting their distinct roles beyond necroptosis. Since RIPK3 also affects lipid metabolism [54], and MLKL influences insulin signaling [72], targeting MLKL may offer a better therapeutic option due to fewer metabolic side effects.

2.5.3. *MLKL inhibitors*

Several reports reveal that necroptosis plays a crucial role in the development and progression of various severe disorders, most notably cancer and inflammation. Many investigators have developed agents that block the MLKL function. A comprehensive knowledge of the mechanisms driving MLKL inhibitors might render it more feasible to develop therapeutic drugs with greater efficacy [73]. P28, a new MLKL inhibitor, has shown significant antifibrotic and necroptosis inhibitory properties. It lowers immune cell mortality, suppresses adhesion molecule expression, and directly inhibits MLKL phosphorylation and oligomerization. The most commonly employed MLKL inhibitor in experimental studies is necrosulfonamide (NSA). It reduces necroptosis in human cells by targeting the Cys86 residue in the N-terminal domain. Additionally, by preventing the formation of disulphide bonds between monomeric MLKL and Cys86 in human MLKL, NSA facilitates the cross-linking of Cys86 to Cys32 in thioredoxin-1, which suppresses necroptosis [74].

MLKL ATP-binding inhibitor, compound-4 (CPD4) lowered the expression of inflammatory mediators in liver organoids and attenuating hepatic inflammation in an ALD model. It exerts anti-inflammatory effects through a necroptosis-independent MLKL pathway [75]. The ATP mimetic SYN-1215, commonly referred to as Compound 1 or GW806742X, is the first well-characterized MLKL inhibitor. SYN-1215 binds to the MLKL activation loop by attaching itself to the nucleotide-binding site in the mouse MLKL pseudokinase domain. Furthermore, the interaction suppresses necroptosis by preventing MLKL from switching its conformation [76]. Other FDA-approved drugs like Pazopanib and Ponatinib have been developed as necroptotic inhibitors through unidentified targets. Both drugs interfere with signaling proteins upstream of MLKL via inhibiting MLKL's phosphorylation during TNFα-induced necroptosis [64].

2.5.4. *Off-target effects and challenges associated with RIPK1, RIPK3, and MLKL inhibition*

Targeting necroptotic factors such as RIPK1 [85], RIPK3 and MLKL [86] in necroptosis pathway presents several off-target effects and challenges in liver aging and chronic liver disease progression. For example, RIPK1 inhibitor, Necrostatin-1 inhibit indoleamine 2,3-dioxygenase (IDO), an enzyme unrelated to necroptosis, raising concerns about specificity [87]. Studies reveal that Nec-1 was initially found to reduce necroptosis in acetaminophen (APAP)-induced liver injury. However, later it was found that Nec-1 can ameliorate necroptosis through other pathways like Notch signaling [88], and NF-κB pathway [89]. In aging liver, RIPK1 inhibition decreases necroptosis markers (p-RIPK1, p-RIPK3, p-MLKL), inflammation, and fibrosis, suggesting therapeutic potential. However, hepatocyte-specific deletion of RIPK1 gene expose liver to various metabolic stress stimuli. This duality underscores RIPK1's scaffold function in maintaining metabolic homeostasis, where kinase inhibition may disrupt physiological balance [90].

The involvement of MLKL and RIPK3 in pathways other than necroptosis is a serious concern. For example, RIPK3 can exert functions independently of necroptosis, influencing other cell death modalities like ferroptosis and potentially affecting inflammatory signaling (Moerke et al., 2019). Similarly, MLKL is now known to regulate inflammation and immunological responses in addition to necroptosis, regardless of its involvement in cell death. Accordingly, suppressing these kinases may inevitably disrupt the physiological functions or trigger alternative cell death pathways like ferroptosis or apoptosis, which could exacerbate inflammation or tissue damage [73]. Another major hurdle is the context-dependent effects of MLKL and RIPK3 inhibition. For example, single knockouts of either gene in mice have protection from necroptosis-driven diseases; but, when both genes are knocked out together, these advantages are paradoxically eliminated,

leaving the animals just as vulnerable as wild-type controls (Moerke et al., 2019). The dual roles of RIPK1, context-dependent outcomes, pathway redundancy, and off-target effects complicate the clinical translation of selective inhibition of necroptotic markers (RIPK1, RIPK3, and MLKL) which hold promise for regulating necroptosis in liver diseases and possibly in aging.

Therapeutic strategies targeting necroptosis in liver aging remain in the early preclinical stages, with ongoing research focused on refining approaches for its clinical utility. While promising results have been observed in experimental models, where blocking necroptosis reduced liver inflammation and slow down disease progression, the translation of these therapies to clinical practice is still a challenge. The future of necroptosis-targeted therapies lies in the development of selective inhibitors that can modulate the pathway without disrupting other essential cellular processes.

2.6. Concept of targeting necroptosis in liver aging and its translational potential

Presently, therapeutic agents that inhibit necroptosis in liver aging-related dysfunctions are still in the pre-clinical stage and none of them have received clinical approval. Recently reported animal model studies have claimed that necroptosis, prominently mediated through RIPK1, RIPK3, and MLKL, is upregulated in aged livers and contributes to chronic inflammation, fibrosis, and hepatic injury [5]. Pharmacological agents like Necrostatin-1s (Nec-1s) targets RIPK1 molecule and have exhibited promising effect in ameliorating necroptosis-related inflammation in aged livers of mice [77]. Some FDA-approved anti-cancer drugs (e.g., Sorafenib, Dabrafenib) have anti-necroptotic properties, but their use in liver aging is not established [78]. Moreover, novel approaches like *young plasma therapy* have potential to attenuate the hepatic inflammation and injury through transfusion of young plasma in rodents and supress necroptosis and NLRP3 inflammasome activation [79]. However, these findings are limited to the pre-clinical evidence and the mechanistic pathways, safety, and ethical considerations of such interventions in humans remain unresolved.

Several small molecule inhibitors have discovered and patented, including the MLKL inhibitor necrosulfonamide, multiple generations of Necrostatins (such as Nec-1, Nec-2) that inhibit RIPK1, and GSK′840, GSK′843. Another GSK′872, which inhibit RIPK1 and RIPK3 [80]. Hence, investigating the molecular mechanisms of necroptosis and exploring the therapeutic potential of bioactive compounds have been the keen interest of the current research strategies which can provide a targeted, safe, and ethically viable treatments for age-related liver diseases Moreover, natural products like Kongensin A were found to have an inhibitory effect on necroptosis [81]. However, to date, the safety and efficacy of treatments that specifically target necroptosis in liver aging have not yet been validated in human clinical trials. These findings claims crucial translational research is required before such strategies can be evaluated for clinical use. While long-term safety and efficacy assessments are still necessary, pre-clinical data do support the potential of necroptosis inhibitors, including repurposed agents like Sorafenib, as therapeutic candidates for age-associated liver disorders.

3. Conclusion and future perspective

Liver aging is a complex process characterized by a gradual reduction in liver function, decreased ability to regenerate, and increased susceptibility to liver diseases. Reports indicate that multiple regulated cell death mechanisms like apoptosis, necroptosis, pyroptosis, and ferroptosis drive the age-related liver dysfunction. Necroptosis, a controlled type of programmed cell death, significantly influences the development of various chronic liver conditions such as MASLD/MASH, fibrosis, and HCC. This pathway is propelled by crucial molecules like receptor-interacting protein kinases and MLKL. Activation of these proteins induces cell death and inflammation, thereby exacerbating liver damage and accelerating disease progression. Necroptosis has emerged as a central mechanism in aging livers, characterized by the activation of RIPK and RIPK3 and the phosphorylation of MLKL. This cascade promotes the release of pro-inflammatory cytokines (such as TNF-α and IL-6) and DAMPs, contributing to a sustained inflammatory environment. This chronic, low-grade inflammation, often referred to as "inflammaging," is both a consequence and driver of necroptosis in the aging liver.

Targeting necroptosis presents a viable therapeutic strategy for chronic liver diseases by blocking key elements of this pathway to mitigate inflammation and tissue damage. Necroptosis inhibitors are novel compounds that frequently interact with pharmacological agents used in geriatric individuals. In elderly patients' frequent intake of medications raises the possibility of negative side effects and drug interactions. The pharmacokinetics and pharmacodynamics properties of necroptosis inhibitors may be impacted by age-related alterations in hepatic and renal drug metabolism as well as elevated blood-brain barrier permeability [82]. More research is needed to ascertain the safety, efficacy, and optimal dosage of necroptosis-targeting treatments in older adults, as the majority of these treatments are still in preclinical or early clinical development. Particular care must be taken when designing trials for the elderly, including careful comorbidity assessment, medication review, and efficacy and adverse event monitoring [83]. In addition, ongoing research focus on identifying specific inhibitors of necroptosis that could be used clinically to manage and potentially reverse chronic liver damage. A deeper understanding of necroptosis mechanisms enables researchers to identify more effective interventions for age-related liver diseases and other conditions driven by necroptosis in the liver.

In the context of liver aging, future research should focus on delineating the specific roles and mechanisms of necroptosis that contribute to the decline in hepatic function and increased disease susceptibility in the elderly. Hence, investigating how necroptosis interacts with other age-related cellular processes, such as oxidative stress and mitochondrial dysfunction, could provide deeper insights into the complex biology of liver aging. Moreover, longitudinal studies to evaluate the efficacy and safety of necroptosis inhibitors in aged liver models. This would be crucial to ensure that these treatments can effectively slow or reverse age-related liver degeneration. Additionally, exploring the genetic predispositions that influence the regulation of necroptosis in aging populations could identify at-risk individuals and lead to personalized therapeutic strategies. Overall, by focusing on these areas, researchers can develop interventions that specifically target the age-related aspects of liver disease, potentially leading to breakthroughs in the management and treatment of liver conditions in the aging population.

CRediT authorship contribution statement

Bhagyalakshmi Nair: Writing – original draft, Visualization, Software, Resources, Methodology, Formal analysis, Data curation. **Anjana Menon:** Writing – original draft, Resources, Methodology, Investigation, Data curation. **Marva Abdul Khader:** Writing – original draft, Resources, Methodology, Investigation, Data curation. **Gautam Sethi:** Writing – review & editing, Visualization, Validation, Supervision, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Lekshmi.R. Nath:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Consent for publication

All the authors have read and approved the article.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

Authors did not have any conflict of interest.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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